

**University of Groningen**

## **Functional architecture of higher plant photosystem II supercomplexes**

Caffarri, Stefano; Kouril, Roman; Kereiche, Sami; Boekema, Egbert J.; Croce, Roberta

*Published in:*  
EMBO Journal

*DOI:*  
[10.1038/emboj.2009.232](https://doi.org/10.1038/emboj.2009.232)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2009

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Caffarri, S., Kouril, R., Kereiche, S., Boekema, E. J., & Croce, R. (2009). Functional architecture of higher plant photosystem II supercomplexes. *EMBO Journal*, 28(19), 3052-3063.  
<https://doi.org/10.1038/emboj.2009.232>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

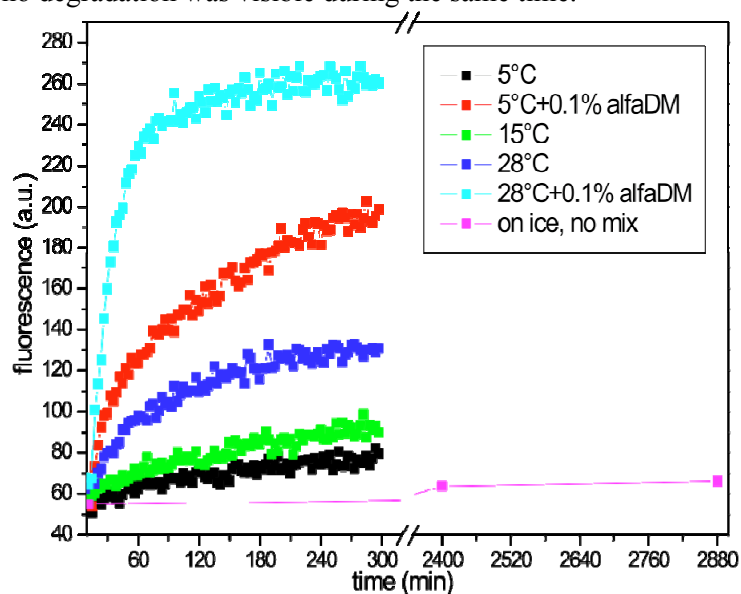
*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

### Figure S1. Supercomplex stability analyzed by steady state fluorescence.

The main problem in obtaining homogeneous preparations of PSII-LHCII is related to the low stability of the supercomplexes. However, a stable preparation is obligatory to be able to investigate the structural and functional characteristics of the supercomplexes. To check the stability of our preparations, we performed steady-state fluorescence measurements in varying conditions. In the presence of very weak light and ferricyanide, the fluorescence yield of intact photosystems is very low due to the fast energy trapping by the reaction centers which remain in the open state. However, if for some reason the antenna complexes detach from the core, the fluorescence of the sample must increase significantly due to the very high fluorescence yield of isolated Lhcb complexes. We performed experiments varying the temperature and/or the detergent concentration in the sample and following the fluorescence changes over time. The results showed that all PSII supercomplexes purified from the gradient were very stable in cold condition (for days on ice and at least several hours at 10°C), while at room temperature some degradation was visible after a few minutes. Increasing the detergent concentration reduces supercomplex stability, as expected, and the effect was particularly fast at room temperature.

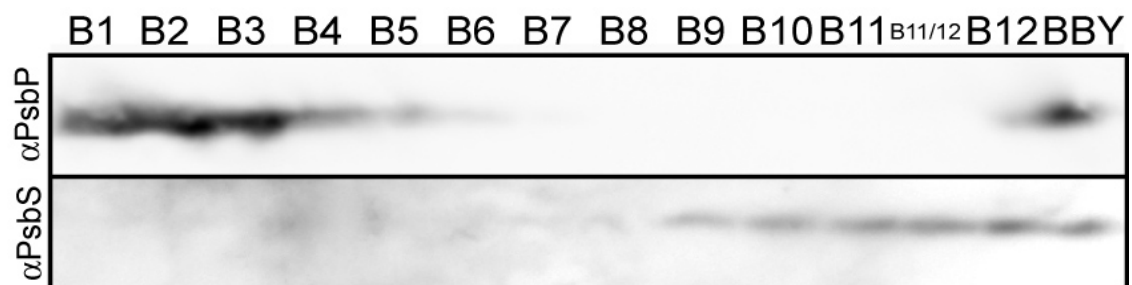
Similar conclusions can be drawn from the supercomplex isolation procedure: if the procedure is not carried out entirely in cold conditions, the concentration of the highest molecular weight complexes (B10 and especially B11) decreases significantly. This suggests that the binding of the LHCII M-trimer, which is attached less strongly than the S-trimer, is particularly sensitive to the temperature.

Fluorescence measurements for stability essays were realized with a Cary Eclipse fluorimeter. To keep PSII reaction centers open during the measurements, ferricyanide 0.3 mM was added to the solution and a very weak excitation was provided. To this aim both a 3% T filter and an excitation in a very low chlorophyll absorbing region (590 nm, 5 nm bandwidth) were used. Emission was detected at 681 nm with 20 nm bandwidth. Samples were diluted to about 0.05 OD in the red peak. Fluorescence increase is indicative of antenna detachment from the core. The kinetics for the biggest supercomplex purified from the gradient (B11: C2S2M2) is shown during 5 hours at different temperature and detergent conditions under gentle mixing with a microstirrer (100 RPM) and for 2 days on ice without mixing. Supercomplexes are very stable for many hours on ice, stable in cold condition in the sucrose gradient solution (5°C or 15°C), while at room temperature some degradation was visible after few minutes. Addition of 0.1%  $\alpha$ -DM detergent reduces supercomplex stability, as expected, especially at room temperature. Note that the small degradation at 5°C is likely mainly due to the mechanical action of the stirrer, since in similar conditions (on ice) without agitation almost no degradation was visible during the same time.



**Figure S2. Western blotting analysis of PsbP and PsbS.**

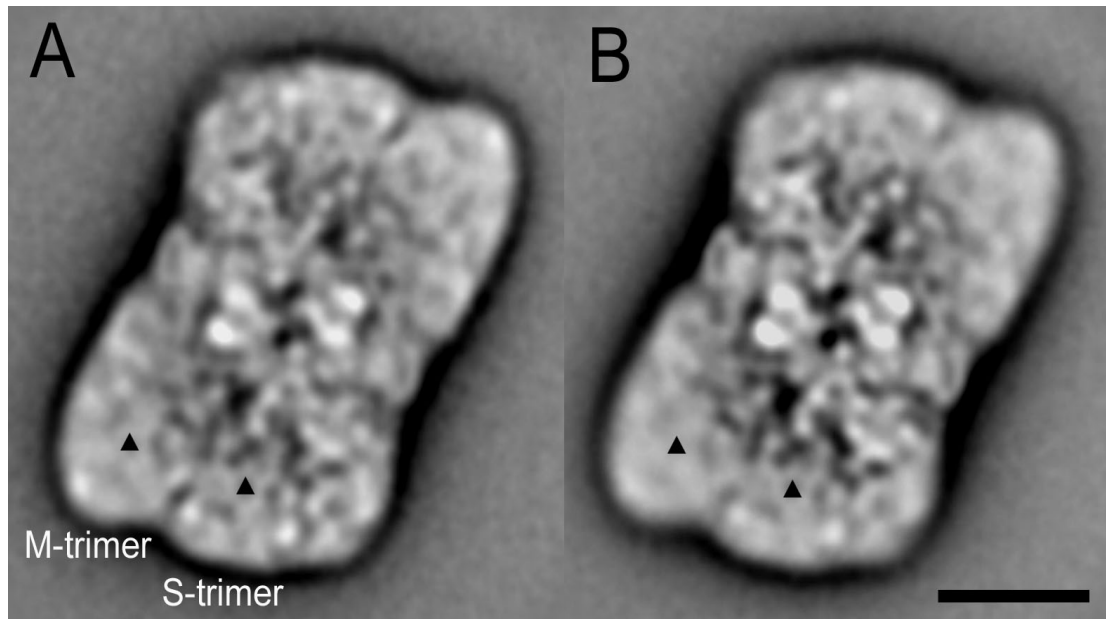
To be able to compare the content of PsbP and PsbS in the different fractions of the sucrose gradient, equal volumes of each fraction were loaded in the gel (10  $\mu$ l). Immunoblotting analysis reveals that PsbP is present in high amount in the first three fractions and in smaller amount in B4 and B5 suggesting that this subunit is detaching from the supercomplexes during the first hours of ultracentrifugation. This can also explain the apparent high amount of PsbP in B5 observed in the SDS-PAGE, which has been concentrated much more than other fractions in order to load a similar amount of Chls in the gel. PsbP is thus likely only co-migrating with B5 but is not functionally attached to the monomeric core. PsbS is present in several fractions, including the fraction between B11 and B12 (B11/12) where a clear decrease in the PSII subunits is evident, but not that of PsbS, thus suggesting co-migration of this protein with the supercomplexes and not specific binding.



**Figure S3. Analysis of the C<sub>2</sub>S<sub>2</sub>M<sub>2</sub> supercomplex of photosystem II.**

(A) a projection map at about 12 Å shows the exact positions of S- trimers and M-trimer of the LHCII; the triangles indicate the position of the three-fold symmetry axis in the center of the trimer. (B) A projection map, focused on improving the center of the supercomplex plus the S-trimer region. In this map, these areas have been slightly sharpened, but at the cost of the M-trimer. Space bar equals 100 Å.

Because we used a small pixel size of 2.3 Å, a maximal resolution of 7-9 Å could in theory be achieved. This resolution, however, was not achieved. One possible reason is the fact that particle alignment tests clearly indicate that the supercomplex structure is slightly flexible in the antenna part occupied by the M trimer.



**Figure S4. SDS-PAGE and Western blot analyses of thylakoids, grana membranes and total leaf proteins of WT and Lhcb-deficient lines.**

A) 1  $\mu$ g (in Chls) of thylakoids membranes and 0.75  $\mu$ g of grana membranes were loaded on a SDS PAGE to test the accumulation of PsbP in the photosynthetic membranes. As shown in the immunoblotting, PsbP is not detectable in thylakoids membranes of koLhcb3 and koCP24, while the protein is strongly reduced in koCP26 with respect to the WT. B) SDS PAGE on total leaf extracts shows that the PsbP protein is present in all mutant plants. These results suggest that the PsbP binding to the PSII is far weaker in the antenna mutants with respect to the WT thus leading to its total or partial loss during the membrane preparation.

